



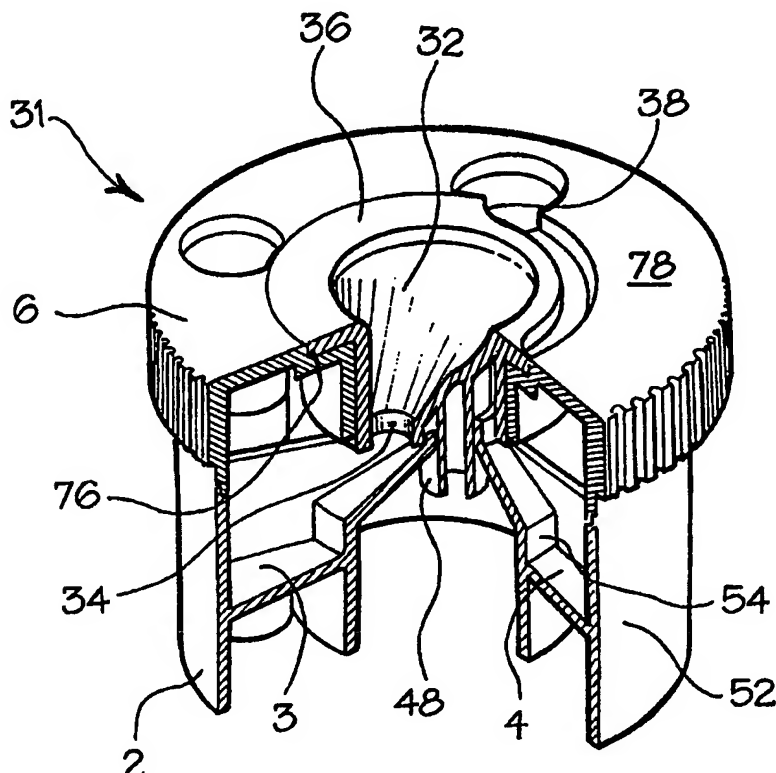
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : B01L 3/00, G01N 21/25		A1	(11) International Publication Number: WO 99/28038
			(43) International Publication Date: 10 June 1999 (10.06.99)
(21) International Application Number: PCT/GB98/03586			
(22) International Filing Date: 30 November 1998 (30.11.98)			
(30) Priority Data:			
9725348.8	28 November 1997 (28.11.97)	GB	
9813292.1	22 June 1998 (22.06.98)	GB	
(71) Applicant (for all designated States except US): CORTECS DIAGNOSTICS LIMITED [GB/GB]; Newtech Square, Deeside Industrial Park, Deeside CH5 2NT (GB).			
(72) Inventors; and			
(75) Inventors/Applicants (for US only): ANDREWES, David [GB/GB]; 5 Ridgeway Road, Farnham, Surrey GU9 8NN (GB). ATTRIDGE, John, Worthington [GB/GB]; Oaksbridge, Send Marsh Road, Ripley, Surrey GU23 6JR (GB). GRIFFITHS, David [GB/GB]; 14 Gordon Road, Claygate, Esher, Surrey KT10 0AQ (GB). VESSEY, John, Phillip [GB/GB]; "Canna", Forest Road, East Horsley, Surrey KT24 5BT (GB). ODELL, John, Anthony [GB/GB]; 24 Halford Road, Fulham, London SW6 1JT (GB). FERNANDO, Felix [GB/GB]; 24 Tudor Close, Wokingham, Berks RG40 2LU (GB). STEVENSON, Anthony [GB/GB]; 4 Beaumont Close, Chester CH4 8PT (GB). CRESSWELL, Mark [GB/GB]; 11 Main Road, Broughton, Chester CH4 0NW			
		(GB). CURTIS, John [GB/GB]; Balderton House, Balderton, Chester CH4 9LF (GB). PERCIVAL, David [GB/GB]; 4 Overlea Drive, Hawarden, Flintshire CH5 3HS (GB).	
		(74) Agent: W.P. THOMPSON & CO.; Coopers Building, Church Street, Liverpool L1 3AB (GB).	
		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
		Published With international search report.	

(54) Title: DEVICE AND APPARATUS FOR CONDUCTING AN ASSAY

(57) Abstract

The present invention relates to an apparatus, instrument and device for conducting an assay. More particularly, it relates to a device suitable for use in assaying analytes, for example glycosylated proteins in biological samples such as, for example, blood. In particular it relates to an apparatus, for use in an assay in which a sample is presented to an instrument, comprising a first inlet, a second inlet, and an inlet port, said inlet port being moveable relative to each of said first and second inlets such that the port can be brought into liquid communication with each inlet in turn as required, wherein said inlet port accommodates a filter means or a binder retaining means. It also relates to an instrument, for reading a sample presented in an apparatus, comprising a microprocessor operable via a key pad, one or more light emitters and one or more light detectors, a display and driver, an analogue to digital converter, and means for connecting the instrument to a power source.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon	KR	Republic of Korea	PL	Poland		
CN	China	KZ	Kazakhstan	PT	Portugal		
CU	Cuba	LC	Saint Lucia	RO	Romania		
CZ	Czech Republic	LI	Liechtenstein	RU	Russian Federation		
DE	Germany	LK	Sri Lanka	SD	Sudan		
DK	Denmark	LR	Liberia	SE	Sweden		
EE	Estonia			SG	Singapore		

- 1 -

DESCRIPTION**DEVICE AND APPARATUS FOR CONDUCTING AN ASSAY**

The present invention relates to an apparatus, instrument and device for conducting an assay. More particularly, it relates to a device suitable for use in assaying analytes, for example glycated proteins in biological samples such as, for example, blood.

The percentage of total haemoglobin (Hb) that is glycated is widely regarded as an important tool in diabetes management, because it provides an indirect measure of the mean blood glucose concentration over the previous 2-3 months. One of the three main methods available for assaying glycated Hb relies on boronate affinity. In this method glycohaemoglobin can be separated from non-glycohaemoglobin through condensation of solid-phase dihydroxyboronate with the *cis*-diols present on the sugar moieties of glycohaemoglobin. This method is specific for all glycohaemoglobins which is an advantage over other methods, which rely on separation based on differences in net charge.

However, although the boronate method has certain advantages, it remains an assay which requires laboratory facilities and quite complicated equipment. In particular, the need to determine the percentage of glycated Hb present means that two assay results need to be obtained and a comparison made. It is the case that rapid diagnostic assays have been developed, and continue to

- 2 -

be developed, which make use of "simple" easy to use diagnostic devices, which can be used either by a subject in their own home, or by a subject's own doctor in the surgery. One example of such a test device is that marketed by Cortecs Diagnostics as HELISAL®ONE-STEP, which is for the detection of *H. pylori* infection. The principle of this device is, however, generally applicable to a range of assays. The device consists of two parts, a sample collector and a second part containing an assay strip. The collector is used to collect a sample (of blood in the case of HELISAL®ONE-STEP) and the collector is then inserted into the second part, with which it interconnects, to release the sample to an assay strip. The sample travels along the strip through various "zones" which contain various reagents, including a coloured label (blue latex particles). If antibodies to *H. pylori* are present then the label concentrates in a detection zone. The specifics of this particular assay are not important, however. The essential features which are common to this type of assay and which allow its use in the home or doctor's surgery are the ease of sample collection and handling as well as the simplicity in initiating the reaction and the speed with which the result is obtained. Such one-step devices can be utilised in the measurement of glycated Hb but only if the assay method can incorporate the necessary sample treatment to allow comparison of total protein with glycated protein.

To that end, therefore, we have in a first aspect of the invention devised an apparatus which allows rapid, easy sample treatment combined with

- 3 -

compatibility with a one-step device such as that exemplified by
HELISAL®ONE-STEP.

According to a first aspect the present invention there is provided an apparatus, for use in an assay in which a sample is presented to an instrument, comprising a first inlet, a second inlet, and an inlet port, said inlet port being moveable relative to each of said first and second inlets such that the port can be brought into liquid communication with each inlet in turn as required, wherein said inlet port accommodates a filter means or a binder retaining means.

In one embodiment the apparatus is adapted to be used in an assay system where some form of particulate is added to a sample which may contain a detectable analyte, where the particulate is capable of binding the analyte. Thus when the sample plus particulate is added to the inlet port, the particulate, with bound analyte, is retained by the filter. The filter can of course be constructed of any suitable material. Suitably, it will be made of material which is inert in terms of the analyte etc. Also the "mesh" of the filter must be such that it is capable of retaining particulates as used in the separation step. The inlet port can then be moved into alignment with the second inlet means and one or more reagents capable of interfering with the binding of the analyte to the particulate can be added to the inlet port. The analyte (if present) will then pass through the filter in solution, leaving the particulates behind.

Thus, taking the example of glycated Hb, a sample of blood is treated to

- 4 -

lyse the blood cells and is then admixed with particulates, eg agarose or cellulose, to which is bound phenyl boronate. The treated sample is then introduced into the apparatus via the inlet port, which will have been moved into liquid communication with the first inlet. The liquid part of the sample, which contains non-glycated Hb, will pass through into the body of the apparatus, while the particulates, to which will be bound any glycated Hb, will be retained by the filter means associated with the inlet port. The inlet port can then be moved into liquid communication with the second inlet and the particulates can be washed with one or more suitable reagents to cause release of the bound glycated Hb from the particulates.

In an alternative approach, the inlet port can incorporate means capable of binding the analyte. For example, it could incorporate particulates such as those described above. Thus, in one embodiment the invention provides apparatus for use in a diagnostic assay, comprising a first inlet, a second inlet and an inlet port, said inlet port being moveable relative to each of said first and second inlets such that the port can be brought into liquid communication with each inlet in turn as required, wherein said inlet port incorporates binding means, capable of binding an analyte which may be present in a biological sample. Such an apparatus would of course also incorporate some means of retaining the binding means in the inlet port.

In preferred embodiments of both the above-described aspects of the invention, the apparatus will also incorporate a third inlet, and the inlet port will

- 5 -

be capable of being moved between the three inlets as required. The third inlet will ideally be placed in an intermediate position between the first and second inlets. The provision of this third inlet will allow for an intermediate washing step to be carried out prior to treating the binding means to release the analyte. In one embodiment the apparatus will be generally circular and the inlet port will form part of a rotatable top portion of the apparatus.

In another embodiment the inlet port will be stationary and the first and second inlets will rotate into communication with the inlet port

As described above the apparatus of the present invention allows a relatively unskilled operative to treat samples, eg blood samples, for assaying in systems such as that used for measuring glycated haemoglobin.

In a preferred embodiment of the above described aspects of the invention the apparatus is designed to be used in conjunction with one-step assay devices such as those described in WO 97/18036. Thus, the apparatus of the present invention can be adapted to allow insertion of one or more sample collectors as described in WO 97/18036. In practice the one or more sample collectors will be inserted such that they are in liquid communication with the first and/or the second inlets. Thus, in use, a first sample collector can be inserted such that it is in liquid communication with the first inlet. In the case of the first aspect described above, the inlet port will also initially be in liquid

- 6 -

communication with the first inlet and the sample plus particulate is added to the inlet port which will retain the particulate, and any bound analyte, allowing the rest of the sample to pass through for collection by the first sample collector.

This sample collector can then be removed and inserted into a test instrument as described in WO 97/18036. The inlet port can then be moved to the intermediate inlet (if present) and wash buffer can be added, flowing through and into a sink incorporated in the apparatus. The inlet port can then be moved into liquid communication with the second inlet and one or more reagents can be added to dissociate the analyte from the particulates. A second sample collector can then collect the analyte solution for removal and insertion into a second one-step device.

Thus, in the case of assays for glycated haemoglobin, the two results obtained can be used to calculate a percentage value for glycated haemoglobin. Conveniently, this can be done using a device such as Cortecs' INSTAQUANT reader which has been designed for use with one-step assay devices.

Suitably, the apparatus of the invention will be constructed of a liquid impervious material such as plastic.

In a more preferred embodiment, the apparatus of the invention is adapted such that the respective samples passing through the first and second inlets are collected in optical chambers disposed below said first and second inlets or said first and second inlets are/or include optical chambers. Thus, in one embodiment the invention provides an apparatus for use in a diagnostic

- 7 -

assay comprising a first inlet, a second inlet and an inlet port, said inlet port being movable relative to each of said first and second inlets such that the port can be brought into liquid communication with each inlet in turn as required, wherein said first and second inlets are in liquid communication with associated optical chambers.

The apparatus is connectable to an instrument which incorporates means for the spectrophotometric measurement of said samples in the optical chambers.

According to a second aspect of the invention there is provided an instrument, for reading a sample presented in an apparatus, comprising a microprocessor operable via a key pad, one or more light emitters and one or more light detectors, a display and driver, an analogue to digital converter, and means for connecting the instrument to a power source.

Preferably each optical chamber houses a micro-cuvette and the instrument comprises means for measuring the absorbance of the contents of each micro-cuvette. Thus, the instrument comprises a LED light source to generate electromagnetic radiation at one side of the sample and an associated photodiode (PD) for measuring the intensity of transmitted light generated across the sample i.e. the instrument measures absorbency. Preferably, the instrument comprises one or more LED/PD pairs. In one embodiment one or more LED/PD pairs are arranged such that when the instrument is connected to the apparatus one or more LED/PD pairs are disposed across each optical chamber.

In another embodiment the apparatus and instrument are connected such

- 8 -

that one or more LED/PD pairs are positioned such that a reading can be taken of a sample in the first optical chamber and then the same one or more LED/PD pairs can be moved to read the sample in the second optical chamber.

Alternatively the optical chambers can be moved relative to the one or more LED/PD pairs.

Producing an instrument with means for the spectrophotometric measurement of said samples proved problematic, since it was necessary to overcome two conflicting problems, namely that:

1. In normal sleep mode, the current drain was only in the order of μ amps, and as a consequence was insufficient to prevent a passivation layer from building up within the electric cell/battery used to drive the instrument, so significant voltage drops occurred when the instrument had not been used for some time; and
2. when running a test, the intermittent loading from the LED's and analogue circuitry was not sufficient to dispose the passivation layer.

In order to overcome these problems it was necessary to:

1. select a lithium thionyl chloride battery;
2. condition it,

(Conditioning can be achieved by for example applying a $1\text{K } \Omega$ load for 24 hours. The skilled man will, however, appreciate that higher loads for shorter period are effective); and

3. regularly switch in a load for a short period of time.

- 9 -

In one embodiment a 3.6V lithium thionyl chloride battery is conditioned by applying a 3.3K Ω load for 7 to 8 hours before soldering the battery onto the main PCB.. This assures that the passivation state of the battery is consistent. The processor is controlled to wake every second by switching in a 1K Ω load for 3.5 mS.

One embodiment of the invention provides an instrument comprising a microprocessor operable via a key pad, one or more light emitting diodes (LED's) and one or more associated photodiodes, a display and driver, an analogue to digital converter, a lithium thionyl chloride battery and a battery conditioning circuit.

The battery is conditioned prior to its incorporation, and soldered, onto a printed circuit board. Conditioning reduces internal resistance in the battery which would result in inconsistent voltages and readings with unacceptable variation.

Circuitry and software is provided to maintain the battery conditioning by repeated discharge of the battery.

Also circuitry and software control systems that energise the LED's in a timed sequence, to permit voltage recovery to stable levels before circuit noise readings are taken and the next reading cycle commenced are provided.

According to a third aspect of the invention there is provided a device comprising an apparatus and an instrument of the invention.

In a particularly favoured embodiment the apparatus of the invention

- 10 -

comprises three main components:

a base portion; a top portion and a funnel portion which serves as the inlet port.

The top portion is connected to the base portion to form a carousel and the funnel portion fits within the top portion such that it can in turn communicate with optical chambers present in the base portion.

The funnel portion has a stem which extends from its centre and serves to connect the apparatus to the instrument. The inlet port funnels the sample and reagents in turn into the respective inlets of the base portion and has an outlet displaced from the centre of the funnel. The outlet is designed to either accommodate a filter means or retain a binding means. Preferably a frit sits within the outlet supported by, for example, a narrowing of the outlet or a flange. The funnel portion further comprises an annular ring which serves as a guide member about which the carousel comprising the top portion and base portion rotate. The annular ring has a cut away or recessed portion thereby allowing tubes, housed vertically in the carousel, to be presented to the user at the appropriate times during the assay procedure. Housing the tubes vertically reduces the size of the apparatus and reduces packaging costs. An inclined ramp disposed on the floor of the instrument upon which the apparatus sits cause respective tubes to be lifted through openings in the top portion as the carousel is rotated on the instrument. The annular ring thus also functions to retain the tubes until they are ready for presentation thus making sure the assay reagents are presented in a correct order.

- 11 -

The top surface of the top portion, as noted above, comprises a plurality of apertures through which respective tubes containing the reagents pass.

The top portion also has an indicator means, which denotes the position for location of the apparatus on the instrument. Preferably, this is in the form of a projecting member which assists the operator to turn the apparatus in the instrument, and more importantly it can be aligned with markers denoting operating positions on the instrument.

The base portion comprises a guide member of a guide pair, which in use co-operate with the other members of the guide pair on the instrument. In a preferred embodiment the base portion has on its side a guide member, for example, in the form of a projecting member which enables the apparatus to be retained and moved in an annular channel in the instrument. The guide member also importantly functions to maintain the optical chambers of the apparatus in a position such that accurate readings can be taken. The base portion comprises a first and second inlet in the form of optical chambers which optical chambers can be rotated with the base portion to be in liquid communication with the inlet port. The optical chambers have a geometry so that the LED's in the instrument can be positioned at the centre of curvature. This has the advantage that all rays in the horizontal plane will be perpendicular to the walls of the optical chamber and should not be subject to refraction. This relaxes rotational location tolerances of the apparatus.

Preferably the optical surfaces of the optical chambers will be recessed to

- 12 -

avoid damage on rotation and prevent a risk of them picking up dirt on handling.

The third inlet which need not be an optical chamber will preferably contain a means for drawing the wash liquid through. Such means might include an absorbent or wicking material such as, for example, filter paper. Other materials such as, for example, acetate based weaves, felts and the like could, however, be used.

Preferably the top and base portions are connected in a manner such that used - reagents are sealed therein. This is most conveniently achieved using a ring seal between the portions.

Preferably, the base unit is made of a clear material, although depending on the application of the apparatus a tinted or coloured material, preferably plastics could be used. Alternatively, an optical filter can be positioned in front of the optical chamber and a white light source used. The optical filter is preferably a wavelength filter.

The apparatus is intended to be disposable.

The apparatus is designed to operate on a ratchet mechanism so that it can only be rotated in one direction on the instrument.

In a particularly favoured embodiment the instrument is run, not from a lithium thionyl chloride battery under the control of a battery conditioning circuit but from an external source, for example, a mains source or car battery via a transformer. Consequently, the apparatus is provided with a power management and monitoring circuit. Preferably the instrument is provided with

- 13 -

a communications system such as, for example, an RS 232 thereby providing means for sending and receiving instructions and down loading data.

The instrument's electronics are housed in a case which is specially adapted for use with the apparatus of the invention. It comprises a recess into which the apparatus of the invention sits. The recess is defined by a floor, an innermost side wall (which is the outer wall of a spigot projecting from the floor) and an outermost side wall. The spigot which projects upwards from the floor of the recess has a portion which mates with a recess in the stem of the funnel portion of the apparatus. Thus, the recess is substantially annular. The outermost side wall has a channel member running about its circumference. This channel is shaped to accept a guide member projecting from the apparatus. This arrangement enables smooth rotation of the apparatus in the instrument and importantly assist in aligning the optical chambers of the apparatus with the light emitter/light detector arrangement of the instrument. The light emitter/light detector arrangement preferably comprises a LED/PD arrangement. The LED's and photodiodes are most preferably arranged such that the reading path of the instrument lies across part of the annular recess. Thus, the innermost and outermost side walls are provided with respective windows through which a path of light from the LED's to the photodiodes can travel. Most preferably the LED's are housed in the outermost wall and the light passes through the optical chamber towards the spigot in which the photodiodes are housed. The LED's and photodiodes could, however, be arranged the other way around. However,

- 14 -

with the former arrangement the convex face of the optical chambers help focus the light giving more accurate readings.

Another feature of the instrument design is a connecting channel running from the top surface of the instrument to the circumferential channel member so as to allow the guide member of the apparatus to be inserted in a set position. Once the apparatus is rotated it is locked in the instrument until it returns to the connecting channel from which it can exit. Also, a ramp is provided on the floor of the instrument's recess so that when the tubes housed in the apparatus contact the ramp as the apparatus is rotated they are lifted presenting them to the user.

The various aspects of the invention will now be described by way of example only, with reference to the following figures in which:

Fig. 1 is a perspective view of an embodiment of the first aspect of the present invention.

Fig. 2 is a block diagram showing the electronics of an instrument of the 4th aspect of the present invention.

Fig. 3 is an embodiment of a device of the present invention.

Fig. 4a is a schematic showing how the device of Fig. 3 is used in an assay;

Fig. 4b is a flow chart showing a protocol for the use of the device shown in Fig. 3.

Fig. 5 is a perspective view of a preferred embodiment of an apparatus of

- 15 -

the invention;

Fig. 6 is a partially sectioned view of the Fig. 5 apparatus;

Fig. 7 is a perspective view of the base portion of the apparatus of Fig. 5 and 6.

Fig. 8 is a perspective view of a preferred embodiment of an instrument of the invention for use with the apparatus illustrated in Figs. 5 and 6; and

Fig. 9 is a perspective view of a preferred device comprising the apparatus as illustrated in Figs. 5 and 6 and the instrument as illustrated in Fig. 8.

Referring to Fig. 1, the apparatus 1 comprises a base section 2 and a rotatable top portion 6. The rotatable top portion 6 itself comprises a handle section 8 and an inlet port 9, the inlet port incorporating a filter means 7. The base section 2 has three inlets 3, 4 and 5 which are associated with three "O" rings 11. A foam pad "sink" 10 is inserted in the middle inlet 4 to collect washing buffer. In this embodiment similar foam pad "sinks" 12 and 13 are associated with the other inlets 3 and 5. The rotatable top portion 6 is retained in place by means of a spring clip 14. Also shown in Fig. 1 are two sample collectors 15 and 16 which can be inserted into the apparatus 1 by way of openings 17 and 18 such that they will be in liquid communication with the inlets 3 and 5.

Thus, in operation, the top portion 6 is first moved to a first position where the inlet port 9 is aligned with the first inlet 3. A first sample collector

- 16 -

15 is inserted in the first opening 17. The sample plus particulate is then added to the inlet port 9, where the particulates will be retained by the filter means 7 allowing the liquid phase to pass through where it is taken up by the sample collector 15. Any excess liquid will be retained by the "sink" 12.

The top portion 6 is then moved to a second position where the inlet port is aligned with the intermediate inlet 4 and wash buffer is added to wash the retained particulates. The wash buffer passes through and is retained by the "sink" 10.

The top portion 6 is then moved to a third position where the inlet port 9 is aligned with the remaining inlet 5. One or more suitable reagents is then added to cause the analyte to dissociate from the particulates and pass through the filter means 7 to be collected by a second sample collector 16 inserted in the apparatus 1 by means of the second opening 18. Each sample collector can be removed and assays carried out in accordance with the principles described in WO 97/18036.

According to a fourth aspect of the present invention there is provided an assay, conducted using an apparatus of the invention wherein a sample is separated into a first component fraction and a second component fraction and the assay determines the presence or absence of one or more analytes in said sample fractions.

As described above, the apparatus of the present invention is particularly suited to use in assays for glycosylated proteins such as glycosylated haemoglobin.

-17-

Thus, in one embodiment the present invention provides an assay for determining the percentage of one or more glyated proteins present in a blood sample, which comprises the step of using an apparatus as described herein to separate a blood sample into a first component comprising one or more non-glyated proteins and a second component comprising one or more glyated proteins.

Preferably, the assay further includes one or more of the following steps:

- (i) obtaining a blood sample from a subject;
- (ii) treating the blood sample obtained in (i) to lyse the blood cells; and
- (iii) providing to the sample obtained in (ii) a means for binding glyated proteins, for example a solid phase to which is bound one or more reagents capable of binding glyated proteins.

Examples of glyated proteins which can be assayed using this method include glyated haemoglobin, glyated human serum albumin and glyated apo lipoprotein B. These proteins will be bound by the boronate ligand and so an assay can be performed in which all three glyated proteins will be bound to a particulate. The component containing all three glyated proteins can then be collected and individual assays can be run to determine the relative amounts of each glyated component. Alternatively, a single one-step device could be used which had three individual capture zones bearing a reagent specific for each of the three glyated proteins. The relative amounts could then be determined using a device such as the INSTAQUANT reader.

- 18 -

An apparatus of the present invention can be included in a kit for use in an assay for one or more glycosylated proteins. Thus, in a further aspect the present invention provides such a kit comprising an apparatus of the invention and optionally one or more sample collectors or one step assay devices or reagents. Examples of suitable one-step assay devices include those described in WO 97/18036, although the skilled person will appreciate that any device designed to allow an assay to be carried by simple addition of a sample to an assay strip can be used.

Another major advantage of the apparatus of the present invention results from the ability to combine a "chemical" or biological capture or separation step, such as the use of the boronate ligand, with an immunoassay or a hand held spectrophotometric means.

According to a fifth aspect of the present invention there is provided a kit comprising an apparatus according to the invention and optionally one or more sample collectors or one step assay devices or reagents and/or a capillary tube and/or an inoculating loop.

According to a sixth aspect of the present invention there is provided a point of care method for the detection of an analyte in a sample which comprises:

(i) separation of the analyte from the sample by the use of chemical or biological means; and

(ii) detection/quantifying the analyte by means of an immunoassay or

- 19 -

spectrophotometric means.

As used herein "chemical" means the use of one or more reagents whose interaction with the analyte is primarily chemical and not biological. For example, as described herein, a boronate based separation step can be used to separate glycosylated proteins from non-glycosylated proteins in a sample. Preferably, step (i) is achieved using apparatus according to the present invention and step (ii) is achieved by means of a one-step assay device.

In a preferred embodiment the apparatus of Fig. 1 is modified to include optical chambers thereby allowing the samples collected to be read spectrophotometrically.

Preferably the discrete optical chambers house micro cuvettes. By measuring the contents absorbance at a given wave length more accurate readings, than can be obtained using reflected light, can be obtained. Thus, preferably the apparatus is adapted to be connected to an instrument with means for measuring the absorbance of the liquid collected in the optical chambers. Figure 2 is a block diagram illustrating the essential components of one such instrument.

Thus, the instrument comprises a body housing a micro processor powered by a lithium thionyl chloride battery under the control of a battery conditioning circuit. Instructions can be transmitted to the micro processor via a key pad and information/instructions presented via a liquid crystal display powered by a LCD driver. The micro processor controls one or more LED's

- 20 -

which pass light of a given wave length (420 - 430 nm in the case of an instrument for reading glycated haemoglobin) across the optical chambers such that absorbed light is measured by photodiodes. The readings are communicated to the liquid crystal display via an analogue digital converter. A micro switch determines that the device (apparatus and instrument) is activated by the correct connection of the apparatus to the instrument. A LED/phototransistor pair is provided to determine when the apparatus has been disconnected from the instrument.

Electronics of the type illustrated in Fig. 2 and controlling software are incorporated as an integral part of the instrument. The device resulting from the connection of the apparatus and instrument is illustrated with reference to Fig. 3. Thus, the device 20 comprises an apparatus 22 similar to the apparatus 1 of Fig. 1 and an instrument 24 which houses the electronics.

Apparatus 22 differs from the apparatus of Fig. 1 in that the inlets (which correspond to inlets 3, 4 and 5 of Fig. 1) communicate with optical chambers in the base 2 of the apparatus. The apparatus and instrument are connected to one another via respective mating members such that a or respective LED/photodiode pairs present in the instrument are situated on either side of the optical chambers or can be presented in turn to said respective optical chambers so enabling absorbance readings to be taken and communicated to the display 26 provided in instrument 24. A key pad 28 is also provided in instrument 24. The top 6 and base 2 of apparatus 22 are designed to include a chamber 30 for

- 21 -

housing one or more components of a kit, for example reagents such as a wash solution and/or buffer and/or elution buffer and/or a capillary tube. The chamber 30 is shown in its open position in figure 3.

Referring to Fig. 4 a protocol for operation of the device is as follows:

- (i) A finger-prick blood sample is collected into a capillary tube and placed into the sample buffer tube which contains a buffer and an amino phenyl boronate (aPBA) agarose affinity matrix. The tube is capped and inverted several times, which washes the blood out of the tube and into the buffer where the red blood cells are lysed thus liberating the haemoglobin.
- (ii) The tube is left for approximately 60 - 90 seconds, with occasional inversion, during which the glycated haemoglobin present in the sample binds to the aPBA affinity matrix.
- (iii) During this time, the apparatus 22 which is designed to be disposable, is coupled to the instrument 24. The location of the apparatus to the instrument activates the on switch.
- (iv) After about 60 - 90 seconds incubation, the contents of the sample buffer tube are mixed by repeated inversion and then the entire contents are poured into the inlet port which is located in position 1.
- (v) The liquid contents of the tube drain through a frit or other filter means located at the bottom of the first inlet and collect in an optical chamber in the base of the apparatus 22. The aPBA affinity matrix, however, is too large to pass through the frit and therefore collects in the column at the bottom of the

- 22 -

first inlet.

(vi) The liquid contents collected in the first optical chamber contain the non-glycated haemoglobin present in the original sample, the aPBA affinity matrix collected in the bottom of the inlet port 9 contains the glycated haemoglobin present in the original sample.

(vii) On completion of this first step, the instrument directs the user to progress to stage 2, which is accomplished by turning the top part of the apparatus 22 through 90° and stopping at position 2. Again under the direction from the instrument 24 a specific volume of wash buffer is added to the inlet 2 via inlet port 9 and allowed to drain through. This step is to remove any non-specifically bound non-glycated haemoglobin from the aPBA affinity matrix that may be present from step 1.

(viii) The instrument 24 then directs the user to progress to stage 3 and add the contents of the elution buffer tube to the inlet 3 via inlet port 9 which is allowed to drain through the frit and collects into a second optical chamber in the base of the apparatus 22. The elution buffer removes the glycated haemoglobin from the aPBA affinity matrix.

(ix) The instrument 24 then spectrophotometrically measures the absorbance (at 430nm) of both the non-glycated and the glycated haemoglobin fractions present in the two optical chambers. Using an algorithm built into the instruments software, the % glycated Haemoglobin present in the original whole blood sample is calculated and displayed on the display 26.

- 23 -

(x) The apparatus 22 is disconnected from the instrument 24 and is discarded as biohazardous waste. The instrument is then ready to perform the next test.

More particularly the instrument is controlled to operate in accordance with the protocol outlined with reference to the flow diagram shown in Fig. 4b.

The spectrophotometric measurement of both glycated and non glycated haemoglobin fraction occurs at the interface of the optical chambers of the apparatus with the instrument 24 of the device.

The most preferred apparatus and instrument are illustrated with reference to figures 5, 6 and 8 and together they form a device as illustrated in Fig. 8

Referring to Figs. 5 and 6 the apparatus 31 comprises a base section 2 of clear plastics (shown in detail in Fig. 7), a top portion 6 and a funnel portion 32. The funnel portion 32 is made of a hydrophobic plastics and has a relatively large aperture to simplify emptying of reagents therein. It has an outlet 34 which directs the liquid into the optical chambers 3 and 5 when the apparatus is rotated in an instrument. The outlet 34 includes a frit (not shown) which frit serves to retain particles such as, for example, an amino phenyl boronate agarose affinity matrix. The funnel 32 which serves as an inlet port has an annular rim 36 with a recessed portion 38. The rim 36 partially overlies apertures 40, 42 and 44 formed in the top portion 6 of the apparatus such that tubes vertically disposed in the apparatus cannot pass through the respective apertures until the apertures are aligned with the recessed portion 38 of the

- 24 -

annular rim. Projecting from the underside of the funnel is a stem 48 with a female mating member via which the apparatus 31 is connected to the instrument 24 which has a male member 50 adapted to engage it. The male member 50 holds the funnel in a fixed position relative to the instrument 24 such that the base portion 2 and top portion 6 of the apparatus 31 which together form a carousel rotate around the funnel, the annular rim 36 of the funnel serving as a guide means.

The base portion 6 of the apparatus is made of a clear plastics, is generally annular in shape and is divided into a plurality of compartments. As can be seen from Fig. 7 there are two optical chambers 3 and 5, a third chamber 4, for receiving waste from a wash step, which third chamber is disposed between optical chambers 3 and 5, and three additional chambers 40', 42' and 44' each housing a reagent tube. These chambers 40', 42' and 44', which are disposed below apertures 40, 42 and 44 in the top portion 6 of the apparatus 31, are arranged so that the reagent tubes are presented to the user when the carousel is in the position corresponding to positions IV, VI and VII per Fig. 4a or position 1, 2 and 3 as per Fig. 4b. The optical chambers have a curved outer wall 52 and a curved inner wall 54 of optical quality, which help focus light from the LED's of the instrument 24 through the sample in the chamber to photodiodes at the other side thereof.

Each optical chamber 3, 5 can be brought into liquid communication with the outlet 34 of the funnel inlet port 9. Alternatively, the optical chambers can

- 25 -

be recessed. Extending outwardly from the outermost wall 56 of the base portion 2 is a guide member 58 which sits within a circumferential channel member 60 formed on the outermost wall 62 of the annular recess 64 of the instrument 24. A communicating channel 66 which extends from the channel member 60 in outermost wall 62 to the top face 68 of the instrument 24 allows the guide member 58 to be inserted into the channel member 60 when the apparatus 31 is connected to the instrument 24.

A projecting member or tab 70 on the knurled edge 72 of the top portion 6 acts as an indicator means, denoting the position for locating the apparatus on the instrument and serves to assist in the turning of the apparatus.

The base portion 2 is connected to the top portion and the funnel portion sits in a channel 76 formed by a step on the top surface 78 of the top portion 6.

The instrument illustrated in Fig. 8 has been designed for use with an apparatus as herein before described. In essence it is very similar to the instrument described with reference to figures 2, 3 and 4b. The instrument illustrated with reference to Fig. 8 does, however, differ from that described with reference to Fig 2 in one major way and has a number of novel and advantageously beneficial additional features. Thus, in contrast to the instrument described with reference to Fig. 2 the lithium thionyl chloride battery and battery conditioning circuit is replaced with a power management and monitoring circuit so that the instrument can be connected to, for example, an external dc supply or a car battery. Additionally, the instrument is provided

- 26 -

with a communication system such as, for example, a RS232 thereby providing means for sending and receiving instructions and down loading data.

Significantly, the means for receiving the apparatus is an annular recess 64 in the instrument which is defined by a floor, an outermost sidewall 62 and an innermost sidewall 80.

The floor of the annular recess comprises a ramp 82 on a part thereof. Within the outermost sidewall 62 of the annular recess is a channel member 60 and extending therefrom to the top surface 68 a connecting channel 66.

In use the apparatus is inserted into the annular recess 60 by aligning guide member 58 of the apparatus with connecting channel 66 so that the apparatus is connected to male mating member 50 via its female mating member 48. The guide member 58 can thus enter channel member 60 such that it can be rotated. On rotation a first tube is directed up the ramp 82 and out of its aperture 44 since the recessed portion 38 of the annular ring 36 is aligned with the aperture. In this position the outlet 34 is in liquid communication with the first optical chamber 3 and the first step of the assay described with reference to Figs 4a and 4b can be conducted. By turning the apparatus through a further 90° a wash solution is presented through aperture 42 for use and then on turning the apparatus through a further 90° tube 40, the eluting solution, is presented. In this manner the appropriate reagents are presented for each step of the assay process.

The apparatus and instrument of the invention can be adapted for use in a

- 27 -

number of assays.

In particular the instrument can be modified to read at wavelengths other than the 400 to 500 nm, more particularly 410 to 460 nm, range of the blue LED employed for measuring glycated haemoglobin. Thus, for example coloured light, red, green, yellow etc. LED's or white light and the use of optical filters more preferably wavelength filters could be employed.

Also the apparatus could be modified to make single measurement rather than take several readings as exemplified with reference to the assay described where a percentage figure is calculated from two readings requiring a separation step. Thus, the inlet port and first and second inlets could be replaced by a carousel type apparatus carrying one or a plurality of optical chambers.

The type of assays might, for example, include:

1. ELISA type assays;
2. Affinity chromatography assays; and
3. Chemical analysis of analytes.

Thus, the wave length spread of the instrument could be adapted to measure the two most commonly used ELISA substrates ABTS which is measured at 414 nm and TMB which can be measured at 600nm (blue) or 450 nm (yellow).

Affinity chromatography assays could be used to determine the presence and/or quantify a number of analytes using spectrophotometric analysis by selecting the appropriate wavelength.

- 28 -

Finally, the technology described could be utilised for field testing of chemical analytes. Thus, for example, water and soil analysis in which nitrates or sulphates are calculated or enzyme activity determined are envisaged.

The skilled man will appreciate that the device of the type described herein and its component apparatus and instrument could be used to measure levels of various other analytes in a wide range of samples.

- 29 -

CLAIMS

1. An apparatus, for use in an assay in which a sample is presented to an instrument, comprising a first inlet, a second inlet, and an inlet port, said inlet port being moveable relative to each of said first and second inlets such that the port can be brought into liquid communication with each inlet in turn as required,
wherein said inlet port accommodates a filter means or a binder retaining means.
2. An apparatus as claimed in claim 1 wherein the filter means or binder retaining means is a frit.
3. An apparatus as claimed in claim 1 or 2 wherein said first and second inlets are or include optical chambers.
4. An apparatus as claimed in any of the preceding claims further comprising a third inlet.
5. An apparatus as claimed in claim 4 wherein the third inlet is located between the first and second inlets.
6. An apparatus as claimed in claim 4 or 5 further comprising an absorbent or wicking material in said third inlet.
7. An apparatus as claimed in any of the preceding claims comprising a base portion having a plurality of chambers including said first and second inlet ports,
a top portion which together with the base portion forms a carousel, and

- 30 -

a funnel portion including said inlet port,

said carousel being rotatably mounted about said funnel portion.

8. An apparatus as claimed in claim 7 wherein said funnel portion comprises a means for locating it on or in an optical instrument.
9. An apparatus as claimed in claims 7 or 8 wherein the funnel portion comprises a guide member about which the carousel rotates.
10. An apparatus as claimed in claim 9 wherein the guide member is an annular ring.
11. An apparatus as claimed in claim 10 wherein the annular ring has a cut away or recessed portion
12. An apparatus as claimed in any of claims 7 to 11 wherein the carousel is adapted to house a plurality of tubes in a vertical position and the top portion has a plurality of holes via which the tubes can, in use, exit the apparatus.
13. An apparatus as claimed in any of the claims 7 to 12 wherein the top portion comprises an indicator means.
14. An apparatus as claimed in claim 13 wherein the indicator means is a projecting member.
15. An apparatus as claimed in any of claims 7 to 14 wherein the base portion comprises a guide member of a guide pair, which in use co-operates with the other member of the guide pair on the instrument.
16. An apparatus as claimed in any of the preceding claims wherein the first and second inlets are optical chambers.

- 31 -

17. An apparatus as claimed in any of claims 3 to 16 wherein the optical chambers have curved optical surfaces.
18. An apparatus as claimed in any of claims 3 to 17 where the optical chambers are recessed.
19. An apparatus as claimed in any of claims 7 to 18 when said base portion is made of a clear material.
20. An instrument, for reading a sample presented in an apparatus, comprising a microprocessor operable via a key pad, one or more light emitters and one or more light detectors, a display and driver, an analogue to digital converter, and means for connecting the instrument to a power source.
21. An instrument as claimed in claim 20 wherein the one or more light emitters are LED's and the one or more light detectors are PD's.
22. An instrument as claimed in claim 20 or 21 wherein the power source is a lithium thionyl chloride battery under the control of a battery conditioning circuit.
23. An instrument as claimed in claim 20, 21 or 22 which is adapted to receive one or more sample collectors.
24. An instrument as claimed in claim 23 wherein the sample collector is a carousel.
25. An instrument as claimed in claim 24 wherein said instrument comprises a guide member of a guide pair, which in use co-operates with the other member of the guide pair present on the apparatus.

- 32 -

26. An instrument as claimed in claim 24 comprising a substantially annular recess defined by a floor, an innermost side wall and an outermost sidewall, said outermost side wall comprising a channel member running about its circumference and a connecting channel extending from a top surface of the instrument to the channel member.
27. An instrument as claimed in claims 26 comprising a ramp on the floor of the recess.
28. An instrument as claimed in any of claims 20 to 27 comprising one or more blue LED's.
29. An instrument as claimed in claim 20 comprising one or more sources of white light.
30. An instrument as claimed in any of claims 20 to 29 further comprising an optical filter.
31. A device comprising an apparatus as claimed in any of claims 1 to 19 and an instrument as claimed in any of claims 20 to 30.
32. An assay, conducted using an apparatus as claimed in any of claims 1 to 19, wherein a sample is separated into a first component fraction and a second component fraction and the assay determines the presence or absence of one or more analytes in said sample fractions.
33. An assay as claimed in claim 32 wherein the sample is blood and is separated into a first component fraction containing one or more non-glycated proteins, and a second component fraction containing one or more glycated

- 33 -

proteins.

34. An assay as claimed in claim 33 wherein the one or more glycosylated proteins are selected from one or more of glycosylated haemoglobin, glycosylated human serum albumin and glycosylated apo lipoprotein.

35. An assay as claimed in any of claims 32 to 34 which further includes one or more of the following steps:

- (i) obtaining a blood sample from a subject;
- (ii) treating the blood sample obtained in (i) to lyse the blood cells; and
- (iii) separating the sample into two components using a method which involves binding an analyte to a solid phase to obtain a first component fraction and then releasing the analyte to obtain the second fraction.

36. A kit comprising an apparatus as defined in any one of claims 1 to 19 and optionally one or more sample collectors or one step assay devices or reagents and/or a capillary tube and/or an inoculating loop.

37. A point of care method for the detection of an analyte in a sample which comprises:

- (i) separation of the analyte from the sample by the use of chemical or biological means; and
- (ii) detection/quantifying the analyte by means of an immunoassay or spectrophotometric means.

38. A method as claimed in claim 37 wherein the analyte is a glycosylated protein.

1/9

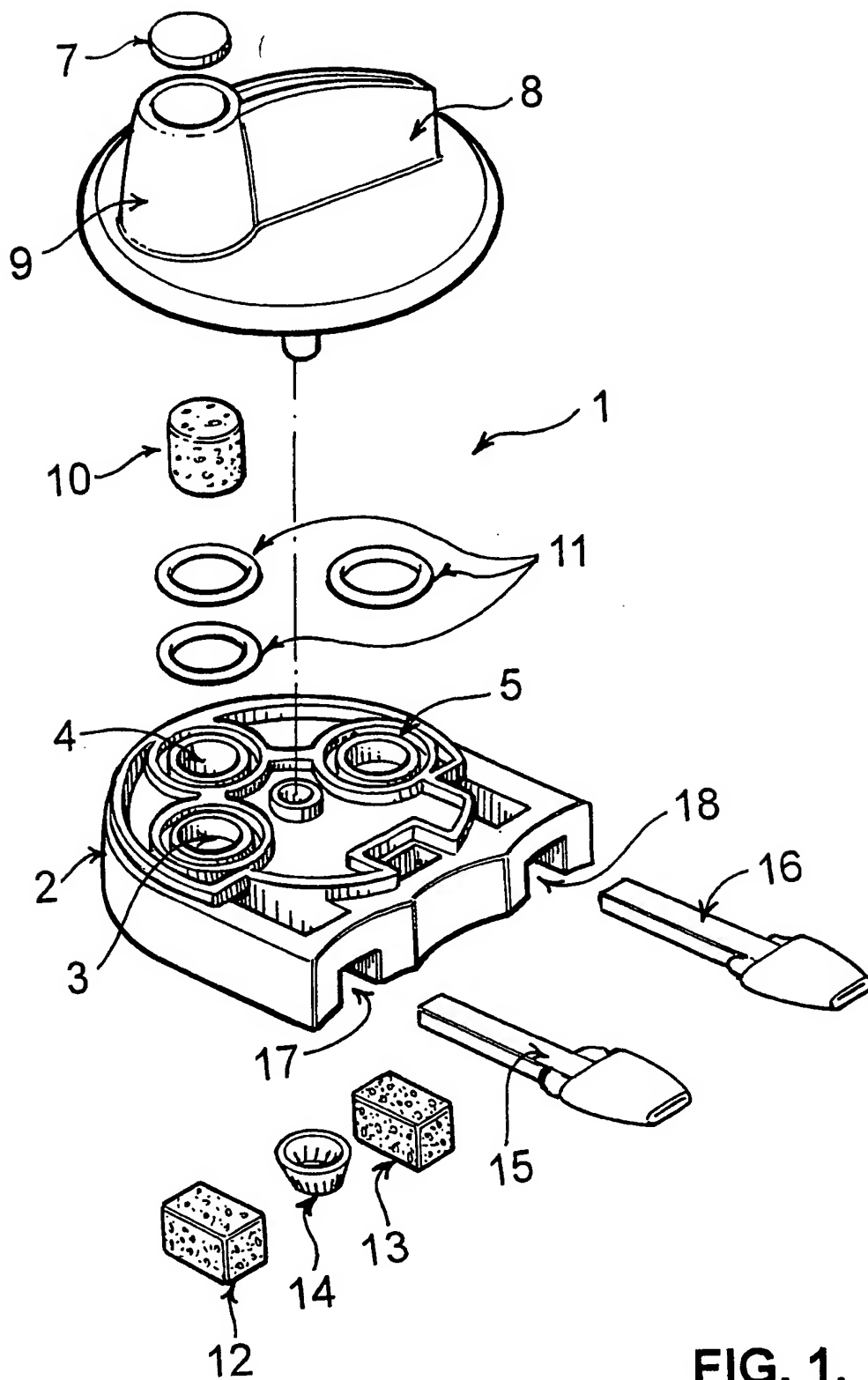


FIG. 1.

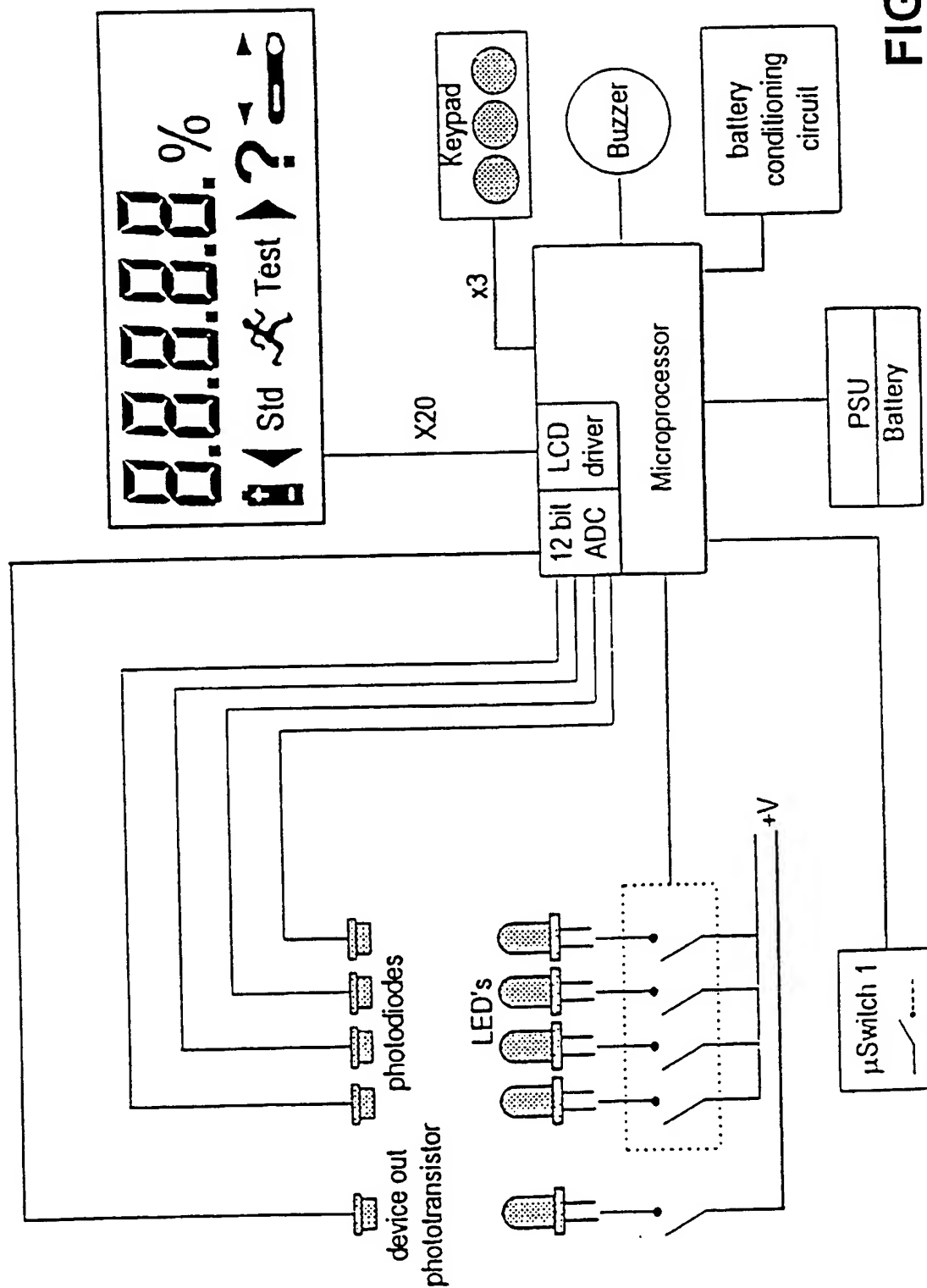


FIG. 2.

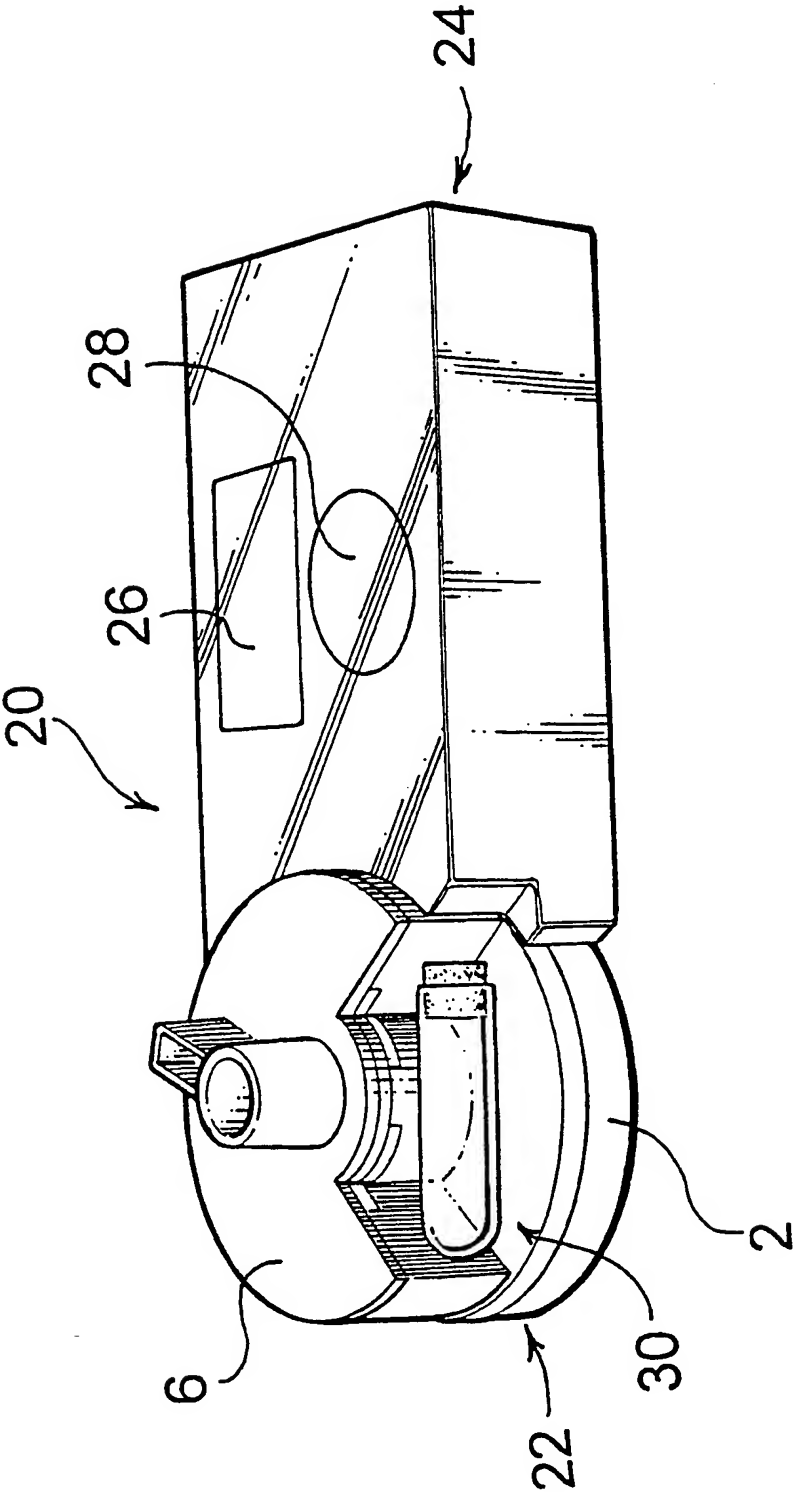


FIG. 3.

4/9

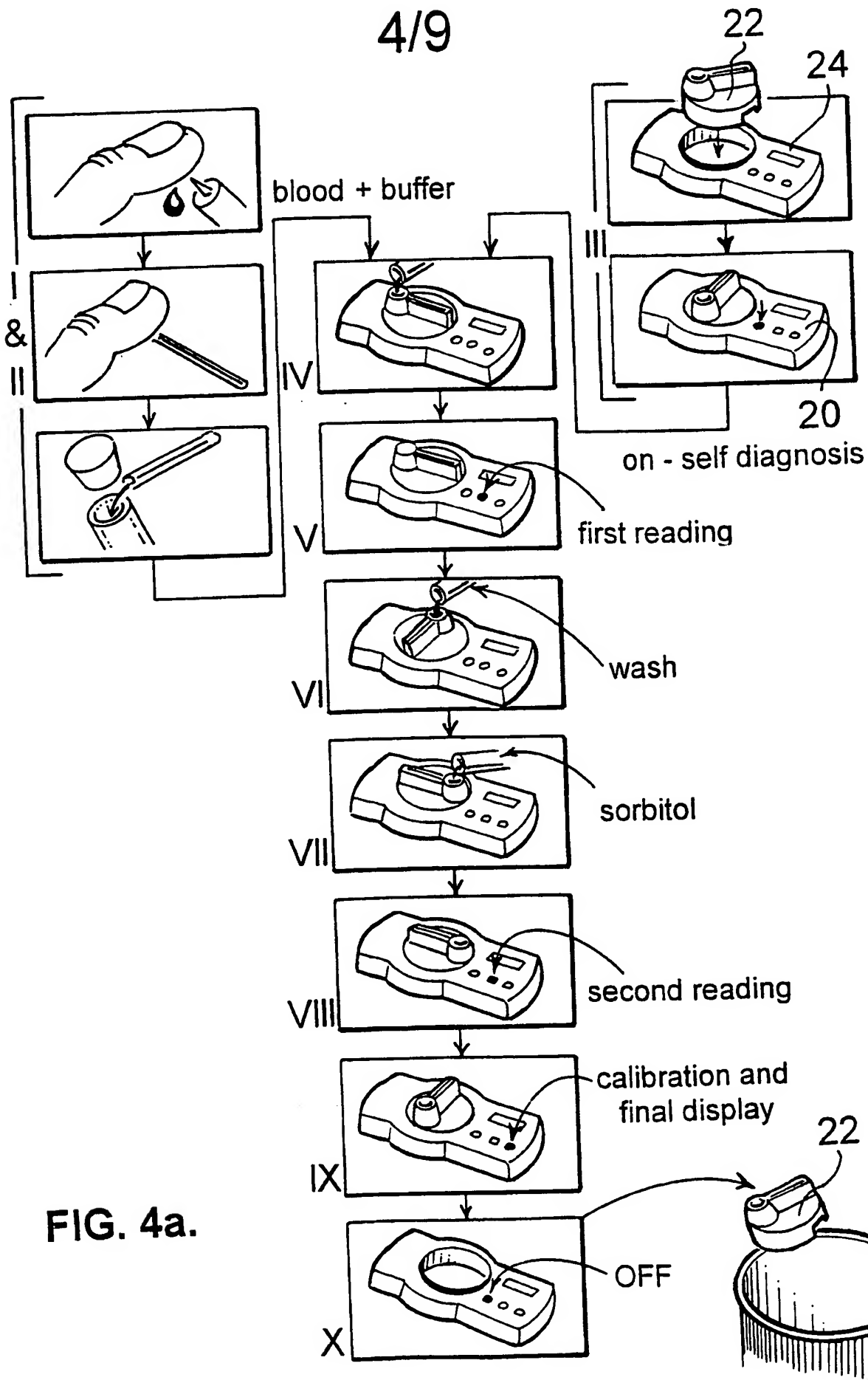


FIG. 4a.

5/9

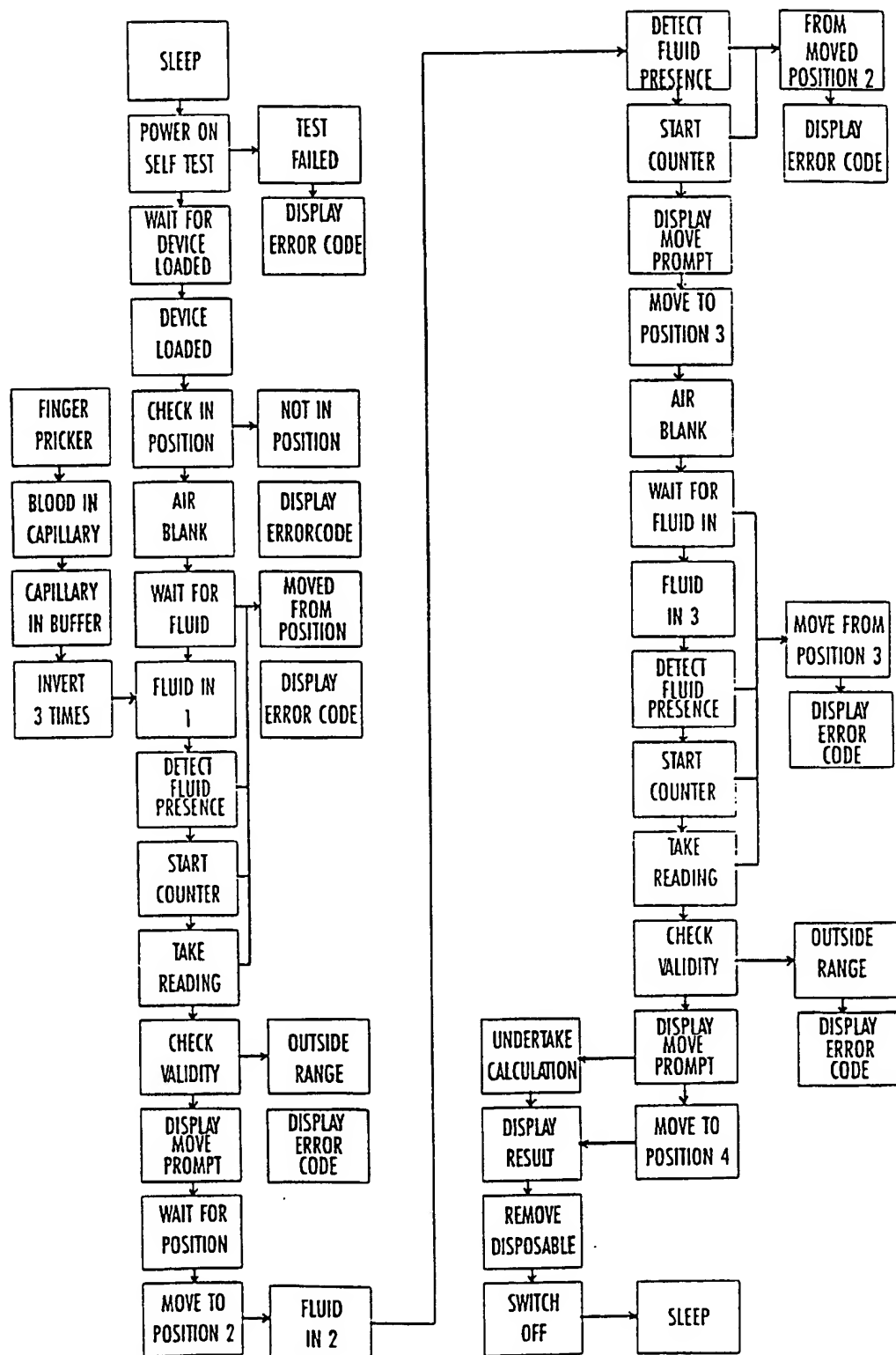


FIG. 4b

6/9

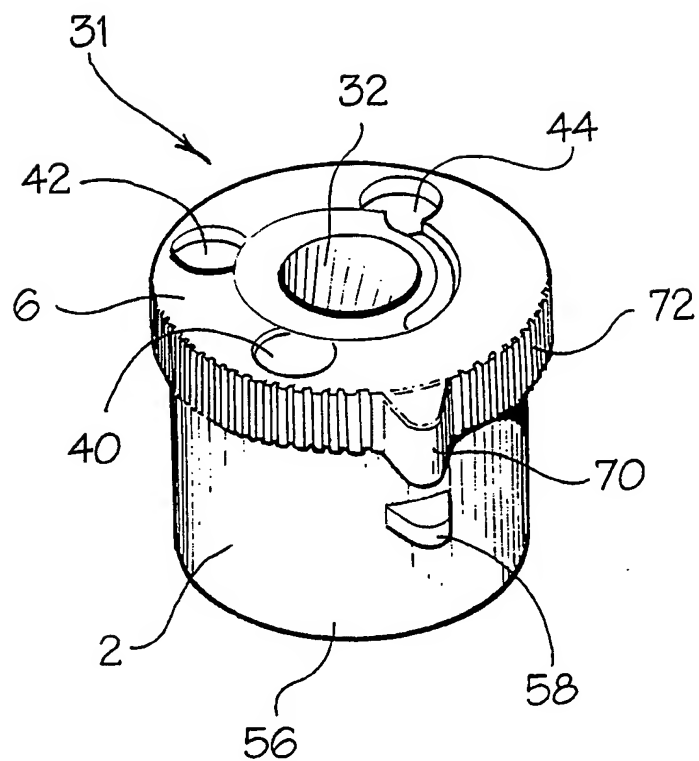


FIG. 5.

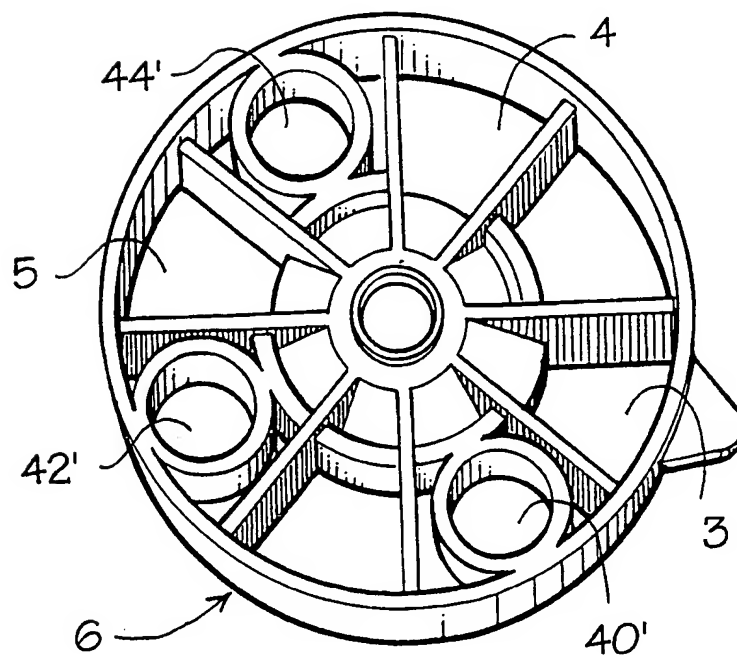


FIG. 7

7/9

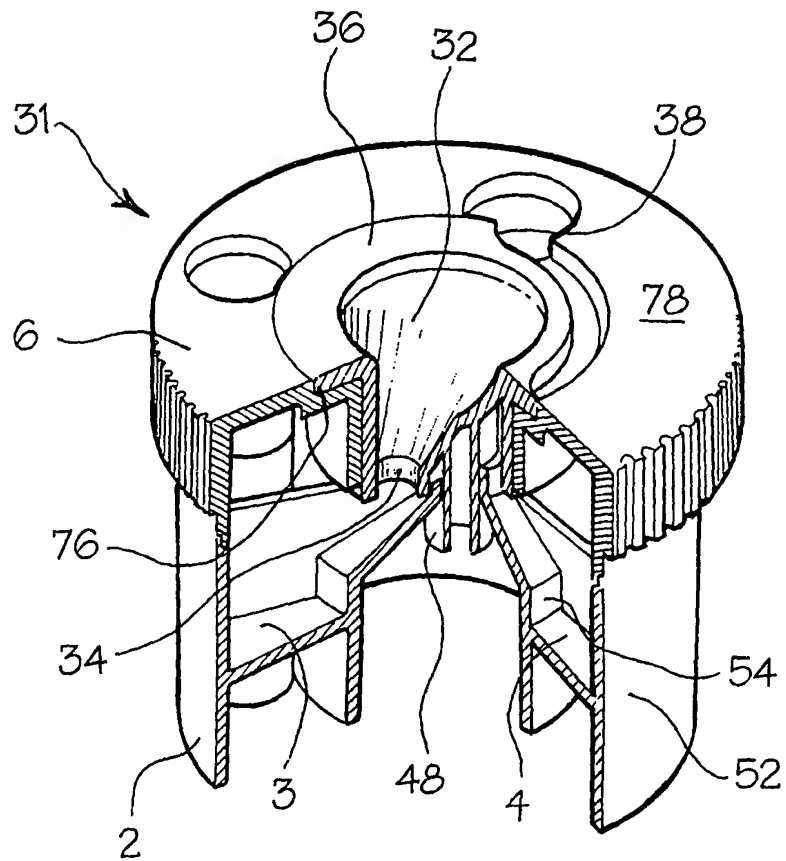


FIG. 6.

8/9

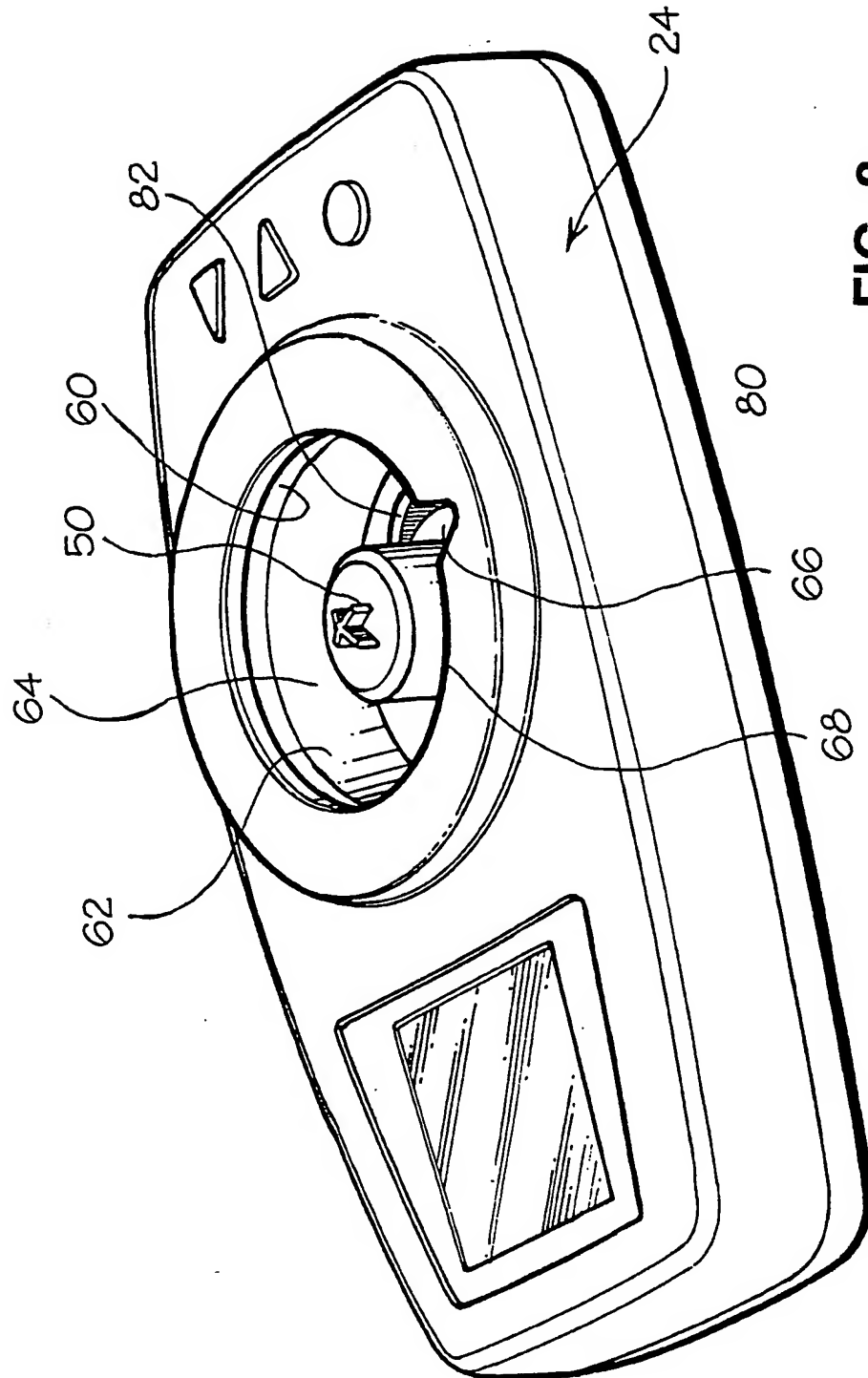


FIG. 8

9/9

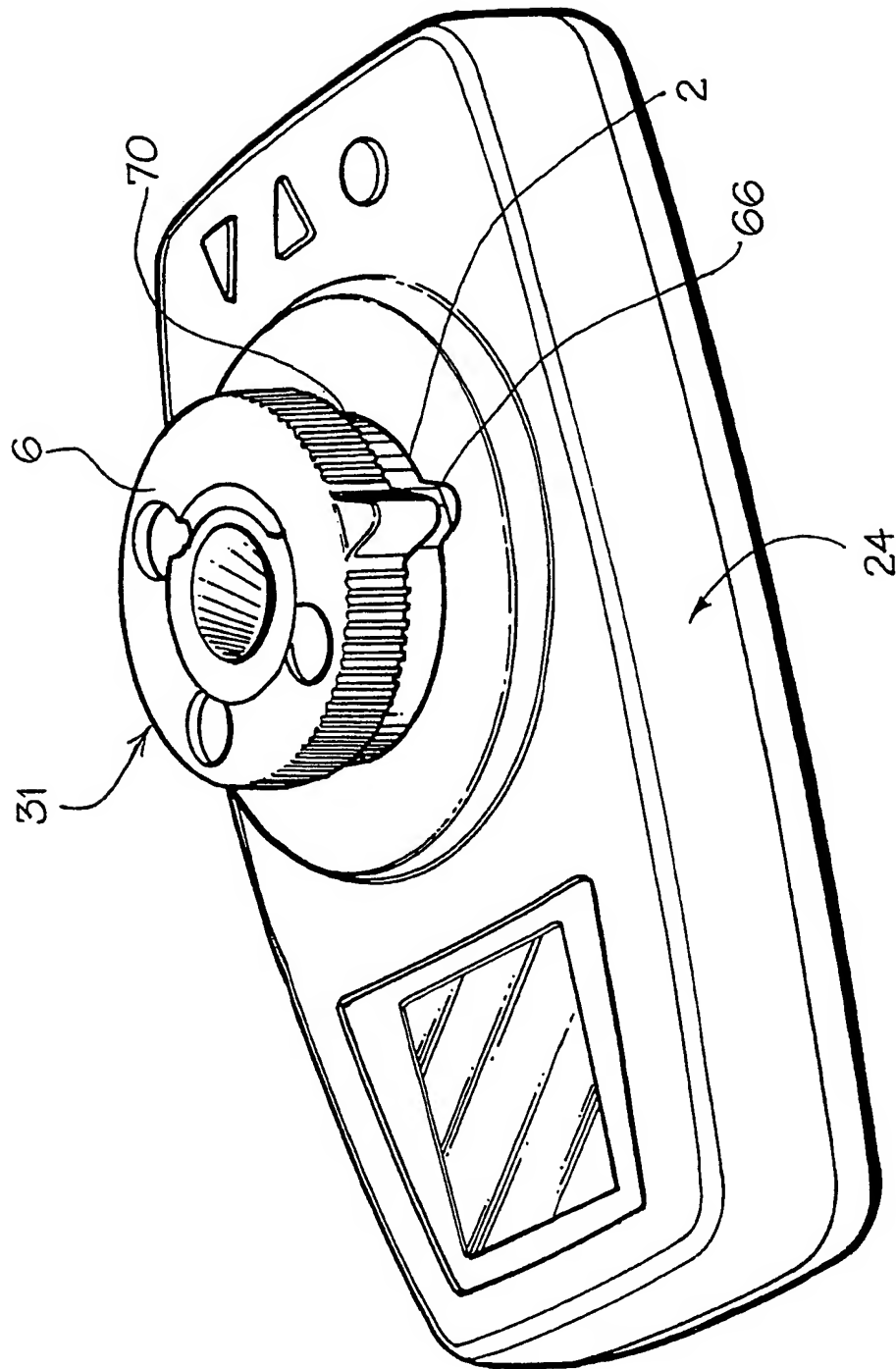


FIG. 9.

INTERNATIONAL SEARCH REPORT

Inte. Application No
PCT/98/03586

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 B01L3/00 G01N21/25

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>EP 0 198 413 A (E.I. DU PONT DE NEMOURS) 22 October 1986 see column 1, paragraph 1 see column 1, paragraph 4 - column 2, line 21 see column 7, paragraph 1 see column 10, line 35 - line 37 see column 12, line 1 - line 38 see column 13, line 43 - column 14, line 1 see column 14, line 13 - line 15 see column 15, line 10 - line 17 see column 15, line 31 - line 33 see column 15, line 49 - column 16, line 1 see column 16, line 15 - line 26 see column 16, line 36 - column 17, line 5 see column 25, line 45 - column 26, line 45 see column 28, last paragraph see figures 1,2,6-8</p> <p style="text-align: right;">-/--</p>	1

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

11 March 1999

Date of mailing of the international search report

19/03/1999

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Thomas, R.M.

INTERNATIONAL SEARCH REPORT

Inter. Application No.
PCT/98/03586

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p style="text-align: center;">---</p> <p>US 3 834 372 A (TURNEY) 10 September 1974 see column 5, last paragraph - column 6, line 13; figures 7-9</p>	1
A	<p style="text-align: center;">---</p> <p>US 4 690 801 A (ANDERSON) 1 September 1987 see column 1, paragraph 1 see column 3, line 14 - column 4, line 49 see figure 1</p>	1
A	<p style="text-align: center;">---</p> <p>EP 0 353 025 A (EASTMAN KODAK) 31 January 1990 see column 2, line 4 - line 21 see column 3, line 42 - column 4, line 26 see column 4, line 47 - column 5, line 34 see figures 2,3A,4,5</p>	1,6
X	<p style="text-align: center;">---</p> <p>WO 91 12515 A (EQM RESEARCH) 22 August 1991 see abstract see page 10, line 2 - line 6 see page 10, line 17 - line 19 see page 11, line 8 - line 12 see page 14, line 12 - line 13 see page 20, line 27 - line 29</p>	20,21,30
Y	<p style="text-align: center;">---</p> <p>see figures 1,2,5</p>	28,29
X	<p style="text-align: center;">---</p> <p>WO 96 31619 A (LXN CORPORATION) 10 October 1996 see page 2, line 10 - line 20 see page 4, line 25 - page 5, line 5</p>	37,38
A	<p>see page 19, line 17 - page 20, line 2 see page 20, line 13 - page 21, line 12 see page 30, line 15 - page 31, line 28</p>	32-35
Y	<p>see figures 3B,4</p>	28,29
X	<p style="text-align: center;">---</p> <p>US 5 402 240 A (THISTLETHWAITE) 28 March 1995 see column 12, line 30 - line 36 see column 13, line 15 - line 17 see column 13, line 48 - line 49 see column 13, line 63 - line 66 see column 14, line 47 - line 48 see column 14, line 54 - line 55 see column 17, line 25 - line 29 see figures 2,3</p>	20
A	<p style="text-align: center;">---</p> <p>WO 97 18036 A (CORTECS) 22 May 1997 cited in the application see abstract see page 15, line 14 - line 17; figure 5</p>	23,25

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter Application No

PCT/CA98/03586

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
EP 0198413	A	22-10-1986	US 4753775 A	28-06-1988
			AT 44620 T	15-07-1989
			CA 1276549 A	20-11-1990
			JP 2582352 B	19-02-1997
			JP 61241664 A	27-10-1986
			US 4806313 A	21-02-1989
			US 4775635 A	04-10-1988
<hr/>				
US 3834372	A	10-09-1974	CA 995560 A	24-08-1976
			DE 2400983 A	18-07-1974
			FR 2326121 A	22-04-1977
			GB 1462391 A	26-01-1977
			JP 49109942 A	19-10-1974
<hr/>				
US 4690801	A	01-09-1987	NONE	
<hr/>				
EP 0353025	A	31-01-1990	US 4877586 A	31-10-1989
			CA 1332911 A	08-11-1994
			JP 2074863 A	14-03-1990
<hr/>				
WO 9112515	A	22-08-1991	US 5073029 A	17-12-1991
			AU 7313791 A	03-09-1991
			EP 0515526 A	02-12-1992
			JP 5504624 T	15-07-1993
<hr/>				
WO 9631619	A	10-10-1996	US 5695949 A	09-12-1997
			CA 2215914 A	10-10-1996
			EP 0819180 A	21-01-1998
<hr/>				
US 5402240	A	28-03-1995	NONE	
<hr/>				
WO 9718036	A	22-05-1997	AU 7577696 A	05-06-1997
			CA 2237395 A	22-05-1997
			EP 0861123 A	02-09-1998
			NO 982156 A	09-07-1998
<hr/>				